

Preparation and antitumoral activity of Au-based inorganic-organometallic nanocomposites

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Submitted to Journal:
Frontiers in Chemistry

Specialty Section:
Nanoscience

Article type:
Original Research Article

Manuscript ID:
428706

Received on:
02 Oct 2018

Frontiers website link:
www.frontiersin.org

Conflict of interest statement

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest

Author contribution statement

A.F., L.R. and I.O.: Designed the experiments; M.D. and A.P. carried out the synthesis and characterization of hybrid systems. P.L.: carried out biological activity experiments; A. F., L.R. and I.O.: Supervised the project. All authors wrote and reviewed the manuscript.

Keywords

Nanoparticles, Gold, Biological activity, Organometallic, Hybrid

Abstract

Word count: 176

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Ethics statements

(Authors are required to state the ethical considerations of their study in the manuscript, including for cases where the study was exempt from ethical approval procedures)

Does the study presented in the manuscript involve human or animal subjects: No

Preparation and antitumoral activity of Au-based inorganic-organometallic nanocomposites

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Abstract

The synergy between gelator molecules and nanostructured materials is currently a novel matter of study. The possibility to carefully design the skeleton of the molecular entity as well as the nanostructure's morphological and chemical features offers the possibility to prepare a huge variety of nanocomposites with properties potentially different than just the sum of those of the individual building blocks. Here we describe the synthesis and characterization of nanocomposites made by the unconventional combination of phosphine-Au(I)-alkynyl-based organometallic gelating molecules and plasmonic Au nanoparticles. Our results indicate that the interaction between the two moieties leads to a significant degree of aggregation in both hydrophilic and hydrophobic media, either when using DAPTA or PTA-based organometallic molecules, with the formation of a sponge-like hybrid powder upon solvent evaporation. The biological activity of the nanocomposites was assessed, suggesting the existence of a synergetic effect evidenced by the higher cytotoxicity of the hybrid systems with respect to that of any of their isolated counterparts. These results represent a preliminary proof-of-concept for the exploitation of these novel nanocomposites in the biomedical field.

Keywords: nanoparticles, gold, biological activity, organometallic, hybrid

Introduction

Metal-organic molecules have attracted much interest in the last decade due to their ability to spontaneously form supramolecular assemblies and metal-organic gels through the establishment of weak non-covalent intermolecular bonds of different nature.¹ In particular, highly luminescent Au(I)-alkynyl-based organometallic complexes have revealed as excellent building blocks for this purpose, forming fibers, gels and other sorts of shape-controlled supramolecular structures, depending on their specific chemical formula and on the reaction conditions, with enhanced optical properties.² Noteworthy, Au(I)-alkynyl-based complexes can form aurophilic intra and intermolecular bonds between Au(I) ions, which do not only reinforce the stability of the assembly, but they also play a crucial role in defining the luminescent properties of the final product.^{3,4}

On the other hand, phosphine-Au(I) compounds have often revealed themselves as cytotoxic species with interesting biological activity against tumor cell growth.⁵ Although most of the studied phosphine-Au(I) metallodrugs contain a thiolate group directly attached to the Au(I) ion, phosphine-Au(I)-alkynyl complexes have been also observed to perform as antitumoral agents in a few cases.⁶⁻⁹ Our group has recently reported some promising results on this topic.¹⁰⁻¹³

In the field of nanoparticles or nanochemistry, Au(I)-alkynyl-based organometallic complexes have been successfully employed as molecular precursors for the synthesis of uniform Au nanoparticles, by means of their controlled thermal decomposition at relatively low temperatures.¹⁴⁻¹⁶ These studies were motivated by the possibility to obtain size and shape homogeneous metallic nanostructures under soft reaction conditions with interesting plasmonic properties. Nevertheless, recent studies are focusing more into the possible synergy established between gelator molecules and different types of nanostructured materials, i.e. inorganic nanoparticles, carbon nanotubes, etc.¹⁷ In this regard, our group has recently reported on the synergy established between phosphine-Au(I)-alkynyl-based complexes and metallic nanoparticles, which allowed for the complete solubilization of hydrophobic colloids into water as well as for their plasmon tuning.¹⁸ Considering the enormous amount of highly designed systems potentially achieved, the preparation of hybrid organometallic nanocomposites should be strongly pursued, since they might open the doors for the use

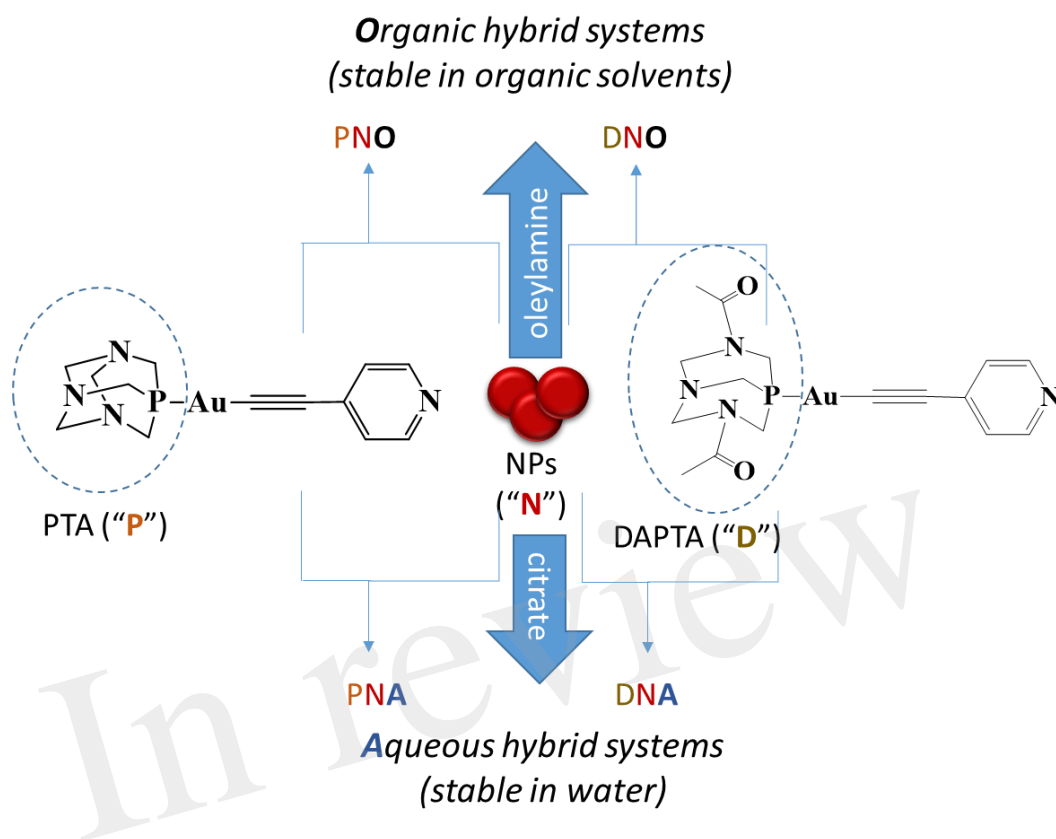
of a new set of engineered materials with multiple and enhanced properties useful in different fields.

Au nanoparticles have experienced a huge growing interest in the last decades due to their fascinating optical properties that are unfolded in the shape of an intense absorption band in the visible or near infrared region (NIR), known as the Localized Surface Plasmon Resonance (LSPR).¹⁹ The origin of this phenomenon in metal nanostructures like Au or Ag is well-understood and is grounded on the collective excitation of surface electrons by means of the electric field of incident light, resulting in a coherent surface-confined oscillation with a resonant frequency that strongly depends on the material itself, as well as on the size, shape, dielectric environment and separation distance of nanoparticles (NPs). The high level of control achieved in their synthesis and morphological features, as well as on their optical properties, have made of Au nanoparticles an interesting material particularly involved in both the photodiagnostics and photothermal therapy of cancers and other main diseases.²⁰

Based on all this, the combination of phosphine-Au(I)-alkynyl-based organometallic complexes and plasmonic nanoparticles seems a promising approach for the design of new theranostic agents, able to perform simultaneously diagnostic and therapeutic tasks when required during the medical treatment. Nevertheless, there are no examples reported on the synthesis of such type of nanocomposites besides our previous work,¹⁸ and thus their potential biological activity is yet to unravel.

In this work, we report on the preparation of novel hybrid nanosystems combining Au nanoparticles and phosphine-Au(I)-alkynyl-based supramolecular structures with the aim of assessing their potential as efficient antitumoral agents, with special emphasis on the identification of synergetic effects between the metallic and the molecular moieties with respect to their isolated counterparts. The antitumoral activity depends on the hydro or lipophilicity of the drug and thus we decided to work with two different types of Au nanoparticles which differ basically on the hydrophilicity of their initial stabilizing molecule after synthesis, being either citrate anions or oleylamine molecules for the hydrophilic and the hydrophobic cases, respectively, as shown in Scheme 1. Our results show that significantly lower IC₅₀ values are found for all the hybrid systems prepared with respect to their individual counterparts (NPs and organometallic structures), representing a good proof-of-concept for the future development and study of these new type of biologically active nanocomposites.

Scheme 1. Types of nanocomposites prepared and description of the nomenclature used for each of them. That is, “N” indicates nanoparticles; “P”, PTA phosphine derivatives; “D”, DAPTA phosphine derivatives; “O”, organic medium and “A”, aqueous medium.



Materials and Methods

General Procedures. Commercial reagents 1,3,5-triaza-7-phosphatricyclo[3.3.1.1^{3,7}]decane (PTA; 97%, Aldrich), 3,7-diacetyl-1,3,7-triaza-5-phosphabicyclo[3.3.1]nonane (DAPTA; 97%, Aldrich), Gold(III) chloride trihydrate ($\text{HAuCl}_4 \cdot 3\text{H}_2\text{O}$, ≥ 99.9 , Aldrich), trisodium citrate dihydrate ($\text{HOC}(\text{COONa})(\text{CH}_2\text{COONa})_2 \cdot 2\text{H}_2\text{O}$, Aldrich), oleylamine (OLAm, 70%, Aldrich), oleic acid (OLAc, $\geq 99\%$, Aldrich), 1-octadecene (ODE, 90%, Aldrich), 2-Propanol (iPrOH, for HPLC, VWR Chemicals) and chloroform (CHCl_3 , 99.2%, VWR Chemicals) have been used as received. Literature methods have been used to prepare $[\text{Au}(\text{C}\equiv\text{C}-\text{C}_5\text{H}_4\text{N})(\text{PTA})]^{21}$ and $[\text{Au}(\text{C}\equiv\text{C}-\text{C}_5\text{H}_4\text{N})(\text{DAPTA})]^{14}$ compounds, that from now on will be named PTA Au(I) complex and DAPTA Au(I) complex, respectively, for the sake of simplicity.

Hydrophilic Au NPs. The synthesis of hydrophilic Au NPs was based on that reported by Turkevich and coworkers.²² Briefly, 8.8 mg $\text{HAuCl}_4 \cdot 3\text{H}_2\text{O}$ (0.02 mmol) were dissolved in 60 mL of deionized water and the solution was heated to the boiling point. Once the temperature was reached, 5.2 mL of an aqueous solution of sodium citrate at 2% was added and let the reaction for 20 minutes at this temperature. The colour of the solution changed from yellow to dark red as a result of the formation of gold nanoparticles.

Hydrophobic Au NPs. The synthesis of hydrophobic Au NPs was adapted from that described by Yu and co-workers as follows:²³ A mixture of ODE (30 mL), OLAc (4.5 mL), and OLAm (5.5 mL) was stirred in a 100 mL three-necked flask for 20 min at 120°C under vacuum. Meanwhile, in a glovebox $\text{HAuCl}_4 \cdot 3\text{H}_2\text{O}$ (120 mg, 0.30 mmol) was dissolved in ODE (7.5 mL) and OLAm (1.5 mL) in an auxiliary vial. Once the purge was complete and the system under N_2 , the solution of gold precursor was injected into the flask and temperature was fixed at 150°C . After 30 min of reaction, the heating was stopped, and the solution was washed with 2 equivalent volumes of iPrOH, and centrifuged at 4500 rpm for 4 min. The product was redispersed in chloroform.

Synthesis of hybrid organometallic supported NPs

Au-DAPTA nanocomposites in organic media (DNO systems). The functionalization of hydrophobic Au nanoparticles with DAPTA was performed by a simple reaction at room temperature in chloroform. 4.2 mg of DAPTA ($7.94 \cdot 10^{-3}$ mmol) were dissolved in 8 mL chloroform and mixed with a solution of hydrophobic Au nanoparticles dispersed in chloroform. The solution was shaken for 24 hours. After this time, the solution was centrifuged and the resultant precipitate was redispersed in chloroform.

Au-PTA nanocomposites in organic media (PNO systems). The functionalization of hydrophobic Au nanoparticles with PTA was adapted from the protocol described above but using 1.3 mg ($2.85 \cdot 10^{-3}$ mmol) of PTA instead of DAPTA.

Au-DAPTA and Au-PTA nanocomposites in aqueous media (DNA and PNA systems, respectively). The synthesis of Au-DAPTA (or -PTA) hydrophilic nanocomposites were done following the same procedure described for the nanocomposites in organic media but substituting the chloroform for deionized water.

Three different samples of each nanocomposite were synthesised using different amounts of Au NPs. The volumes of Au NPs solution used in each case are compiled in Table 1.

Table 1. Volumes of Au NP stock solution used in each synthesis. The concentration of Au NPs in aqueous stock solution was 0.015 μ M and in organic stock solution 2.33 μ M.

SAMPLE	Volume of Au NP stock solution
DNO_1	540 μ L hydrophobic Au NPs (CHCl_3)
DNO_2	270 μ L hydrophobic Au NPs (CHCl_3)
DNO_3	54 μ L hydrophobic Au NPs (CHCl_3)
DNA_1	1600 μ L hydrophilic Au NPs (H_2O)
DNA_2	800 μ L hydrophilic Au NPs (H_2O)
DNA_3	160 μ L hydrophilic Au NPs (H_2O)
PNO_1	540 μ L hydrophobic Au NPs (CHCl_3)
PNO_2	270 μ L hydrophobic Au NPs (CHCl_3)
PNO_3	54 μ L hydrophobic Au NPs (CHCl_3)
PNA_1	1600 μ L hydrophilic Au NPs (H_2O)
PNA_2	800 μ L hydrophilic Au NPs (H_2O)
PNA_3	160 μ L hydrophilic Au NPs (H_2O)

Physical Measurements.

Transmission Electron Microscopy (TEM). Au NPs were prepared for observation by TEM by dilution in chloroform/water followed by sonication. A droplet of the solution was then poured in holey carbon covered copper TEM grids. A JEOL 2000 FX II conventional TEM operating at an accelerating voltage of 80kV was used.

SEM. Scanning electron microscopy (SEM) was carried out at 20 kV using J-7100F (Jeol) equipped with a thermal field electron source.

Infrared Spectroscopy. IR spectra were recorded with a FTIR 520 Nicolet Spectrophotometer. For the measurements, a pellet of a mixture of the sample and KBr was used.

UV-Vis absorbance spectroscopy. A Cary 100 Scan 388 Varian UV/Vis spectrophotometer was used with quartz cuvettes for optical characterization.

ICP-AES. The compositions and concentrations of the nanoparticles solutions were determined by inductively coupled plasma atomic emission spectroscopy (ICP-AES).

The measurements were performed with an Optima 3200 RL PerkinElmer spectrometer. For the measurements, 50 mL of the solutions was precipitated in MeOH and redispersed in CHCl₃. The solution was evaporated in an oven overnight at 90°C. Aqua regia (2.5 mL) and H₂O₂ (0.7 mL) were added to the precipitate before the vial was sealed and then heated to 90°C for 72 h. The resulting solution was transferred to a 25mL volumetric flask and diluted with Milli-Q water.

NMR. ¹H NMR (δ(TMS) = 0.0 ppm), ³¹P{¹H} NMR (δ(85% H₃PO₄) = 0.0 ppm) spectra have been obtained on a Varian Mercury 400 and Bruker 400.

Small-Angle X-ray Scattering (SAXS). SAXS was performed on the NCD-SWEET beamline at the ALBA Synchrotron at 12.4 keV, and the distance sample/detector was 6.2 m to cover the range of momentum transfer of 0.028 < q < 2.56 nm⁻¹. The data were collected on a Pilatus3S 1M detector with a pixel size of 172.0x172.0 μm². The exposure time was 30s. The q-axis calibration was obtained by measuring silver behenate.²⁴ The program pyFAI was used to integrate the 2D SAXS data into 1D data.²⁵ The data were then subtracted by the background using PRIMUS software.²⁶ The maximum particle dimension D_{max} and the pair distance distribution function P(r) were determined with GNOM.²⁷ The low-resolution structure of the aggregates was reconstructed ab initio from the initial portions of the scattering patterns using the program DAMM^{28,29}

Cell Culture and antiproliferative effects

MDA-MB-231 breast adenocarcinoma and HT-29 colon carcinoma were maintained in DMEM high glucose (PAA) supplemented with 50 mg/L gentamycin and 10% (V/V) fetal calf serum (FCS) at 37°C under 5% CO₂ atmosphere and passaged every seven days. Antiproliferative effects were determined as follows: a volume of 100 μL of a 38.000 cells/ml (HT-29) or 40.000 cells/ml (MDA-MB-231) suspension were seeded into 96-well plates and incubated for 48 or 72 hours at 37 °C / 5% CO₂. After the incubation period the cells of one plate were fixed by addition of 100 μL of a 10% glutaraldehyde solution per well. After 30 min. 180 μL PBS were added and the plate was stored at 4 °C (t₀ plate). Nanoparticle suspensions were diluted 1:100 with cell culture medium to the final test concentrations. In the remaining plates the medium was replaced by the medium containing the nanoparticles or solvent (water or CHCl₃) control. Then the plates were incubated for 72 h (HT-29) or 96 h (MDA-MB-231) at 5%

CO₂ / 37 °C. The medium was removed and the cells were treated with 100 µl of a 10% glutaraldehyde solution. Afterwards the cells of all plates were washed with 180 µL PBS and stained with 100 µL of a 0.02% crystal violet solution for 30 minutes. The crystal violet solution was removed and the plates were washed with water and dried. A volume of 180 µL of ethanol 70% was added to each well and after 2-3 h of gentle shaking the absorbance was measured at 595 nm in a microplate reader (Victor X4, PerkinElmer). The mean absorbance value of the t₀ plate was subtracted from the absorbance values of all other absorbance values. The IC₅₀-values were calculated as the concentrations reducing the cellular proliferation in comparison with the solvent control by 50%.

Results and Discussion

Synthesis and Characterization

Our synthetic strategy started by preparing two samples of Au NPs capped by hydrophilic and hydrophobic stabilizing ligands separately, i.e. citrate anions and oleylamine molecules, respectively (see Materials and Methods section). TEM micrographs of the as-prepared samples allowed to evaluate the morphological and size distribution of the NPs in the two samples. The hydrophilic sample consists of a collection of nanoparticles with a significant distribution of shapes including spherical (the most abundant), triangular and elongated nanoparticles of low aspect ratio as shown in Figure 1a. On the contrary, Figure 1b is a TEM representative image of the hydrophobic sample in which only spherical faceted nanoparticles are observed. The size distribution was also much narrower in the hydrophobic sample, even though both samples showed an average diameter of ca. 14 nm and their deposition on the TEM grid suggests that the colloids are well dispersed in solution without evidences of aggregation.

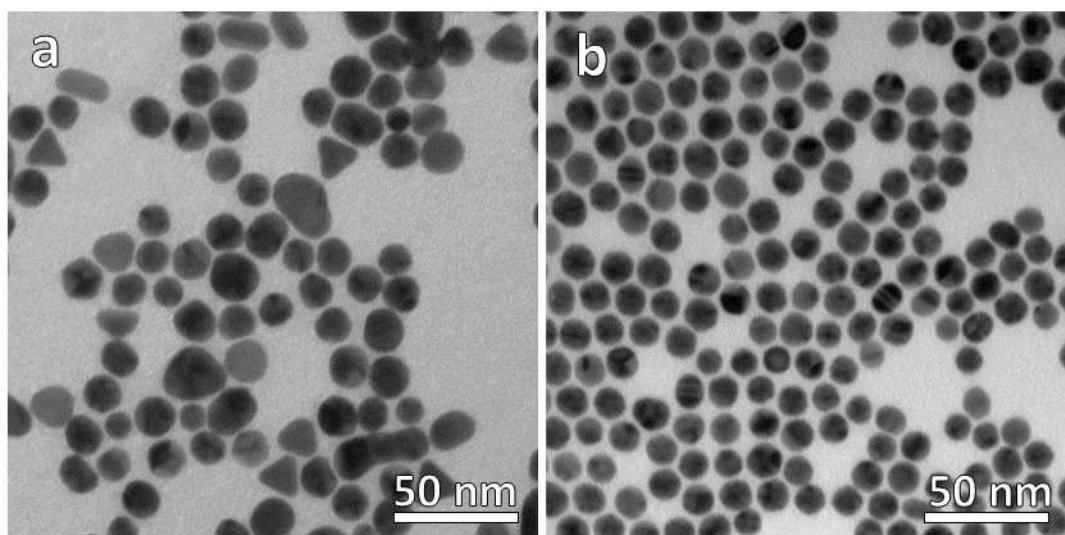


Figure 1. TEM micrographs of (a) hydrophilic citrate-capped and (b) hydrophobic oleylamine-capped Au NPs.

The correct formation of the resulting nanocomposites was evidenced by SEM micrographs as depicted in Figures 2 and 3 corresponding to DNO_1 and PNA_1 samples, made of DAPTA Au(I) complex in chloroform and PTA Au(I) complex in water respectively, and both with the highest amount of Au NPs in the reaction medium. The images suggest that the samples are mainly constituted by aggregates of Au NPs, with sizes ranging between 100 and 300 nm approximately that are well interconnected between them forming a kind of sponge-like 3D network. The individual aggregates are likely surrounded by a shell of the organometallic complex. Indeed, a quantitative shrinkage of the aggregates is evident when comparing topographic images in panels (a) and (c) in the figures, recorded with secondary electrons, with the corresponding Z-sensitive images in panels (b) and (d), recorded with backscattered electrons. The significant reduction in volume of the clusters between the two related images is indicative of the presence of an organic shell around the metallic NPs. Besides the presence of the organometallic-NP composites, few long fibers are also observed at low magnifications (Figures 2c-d and 3c-d). The vanishing of these fibers in images recorded using backscattered electrons confirms their organic origin, suggesting that they are formed as a result of the supramolecular interactions between free molecules of the organometallic moiety, as it was observed in previous studies of these organometallic complexes,¹⁴ and that they do not incorporate Au NPs in their structure.

Generally speaking, the observed trend with amount of Au NPs was the same, independently of the organometallic molecule and solvent used for the synthesis of the nanocomposites: the larger the amount of Au NPs used for the synthesis, the higher the number of composite aggregates encountered, while few or none nanocomposites were found in samples containing the lowest amount of Au NPs (samples XXX_3 of each group), as shown in Figures S1-S4 in the SI. Some large aggregates of approximately 20 μ m can also be appreciated by SEM, although in a much lower frequency compared with the previous smaller ones.

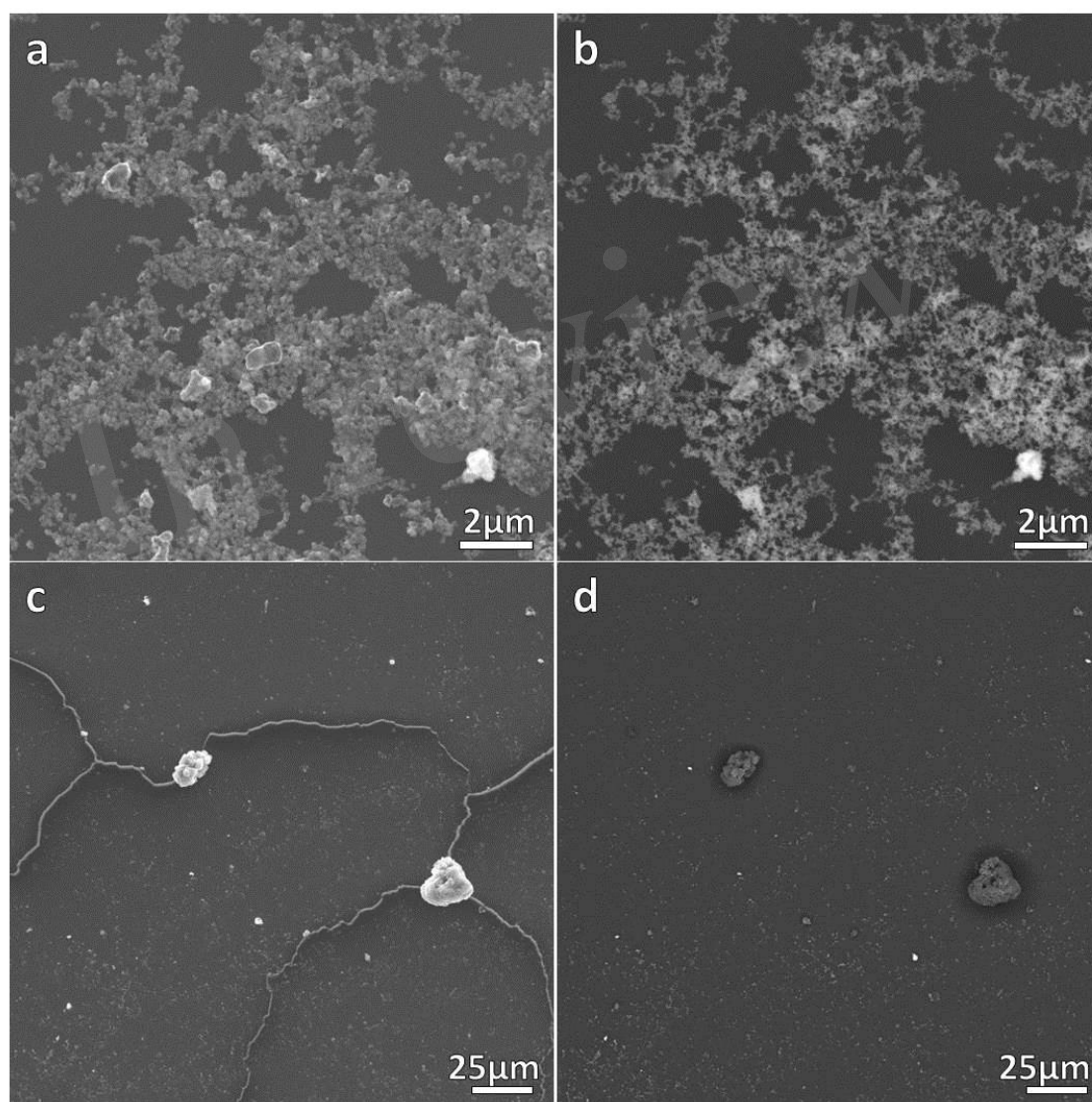


Figure 2. SEM micrographs of DNO_1 sample. Panels (a) and (c) contain topographical micrographs recorded with secondary electrons, while panels (b) and (d) contain Z-sensitive micrographs recorded with backscattered electrons.

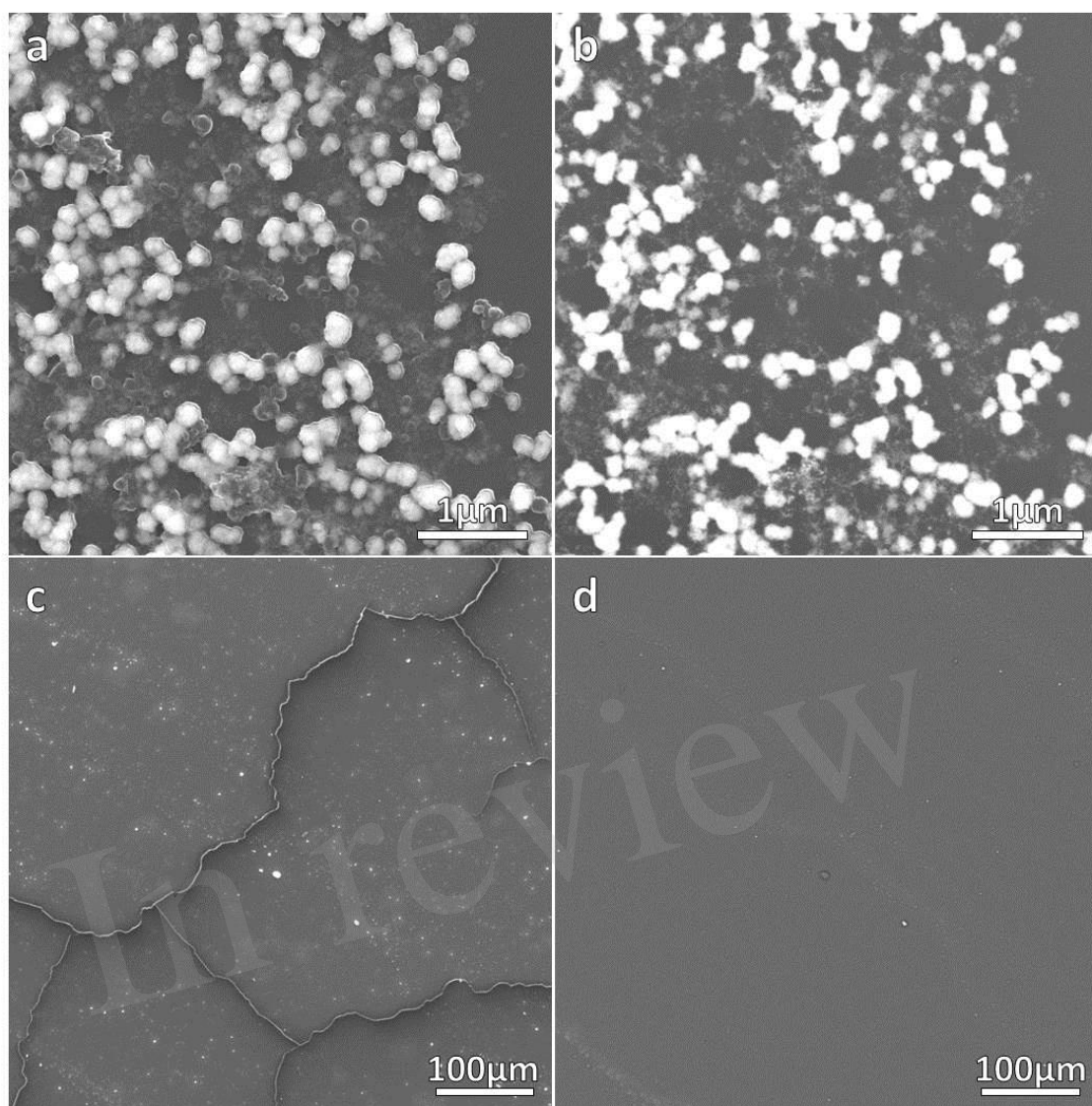


Figure 3. SEM micrographs of PNA_1 sample. Panels (a) and (c) contain topographical micrographs recorded with secondary electrons, while panels (b) and (d) contain Z-sensitive micrographs recorded with backscattered electrons.

The size and shape of the aggregates were measured by SAXS for those samples prepared with the highest (XXX_1) and medium (XXX_2) amount of Au NP. Samples with less amount of Au NPs were not measured since previous SEM studies revealed the presence of no composites in the solutions. Each of the eight considered samples (DNO_1/2; DNA_1/2; PNO_1/2 and PNA_1/2) was measured as initially prepared and also with two additional diluted concentrations (i.e. with DNO_1_1 being the as-prepared sample, and DNO_1_2 and DNO_1_3 the samples derived from 1/500 and 1/1000 dilution), at different temperatures (from 20 °C to 40 °C in a 5 °C gradient) and

one week after their preparation to favor aggregation. The low-resolution structures were reconstructed ab initio from the scattering patterns using the DAMMIN program (see Materials and Methods section). The resulting size and morphologies profile are depicted in Figures 4 and S5-S17 for all compounds that display significant aggregation. No significant aggregation was detected for the rest of the cases. Generally speaking, aggregates of sizes between 10 and 30 nm are measured by SAXS depending on the sample, which hardly agrees with the size of the aggregates observed and measured by analysis of SEM micrographs in Figures 2 and 3. The values obtained by SAXS point out the possibility that the relatively large aggregates observed by SEM do not exist in solution, and that they are only formed upon solvent evaporation during samples preparation. The sizes of the aggregates measured by SAXS are clearly below 100 nm, which seems to suggest that in solution, Au NPs are mainly wrapped within a layer of organometallic complex either isolated one from the other or forming small metallic clusters of a few nanoparticles. Nevertheless, 3D intercluster non-covalent interactions are expected to take place in solution, which are responsible of the sponge-like structure observed after solvent evaporation. In general, it can be seen that temperature does not affect substantially the size of the aggregates. Only in the case of DNO_2, a significant increase on the size is clearly detected. This can be ascribed to the higher contribution of hydrophobic interactions at higher temperatures, being DAPTA moieties (more soluble in water) avoiding the contact with organic solvent. Within each set of samples measured at different concentrations, the collected data indicates that no aggregation occurs for the most diluted samples (XXX_X_3), while appreciable degrees of aggregation were measured for the as-prepared samples (XXX_X_1) and their 1/500 dilution (XXX_X_2). This data could indicate the existence of a critical concentration of aggregates below which they lose their stability. Some other trends can be observed looking at this data regarding now the amount of Au NPs used in the reaction. That is, in organic medium, a light increase of size was detected for PNO_2_X samples containing a smaller amount of Au NPs or aggregates compared to analogous PNO_1_X samples, while on the contrary, DNO_1_X samples give rise to the formation of larger structures compared to the DNO_2_X analogues. These observations could be rationalized based on the lower solubility of DAPTA (in DNO complexes) in organic medium with respect to PTA (in PNO derivatives). Following this reason, it makes sense to understand the contrary effect in water: smaller aggregates for samples PNA_2_X containing a smaller amount of Au NPs or aggregates, and larger

aggregates for samples DNA_2_X containing also a smaller amount of Au NPs or aggregates were observed, since the higher solubility in water of DAPTA Au(I) complex makes the formation of aggregates more difficult in this solvent.

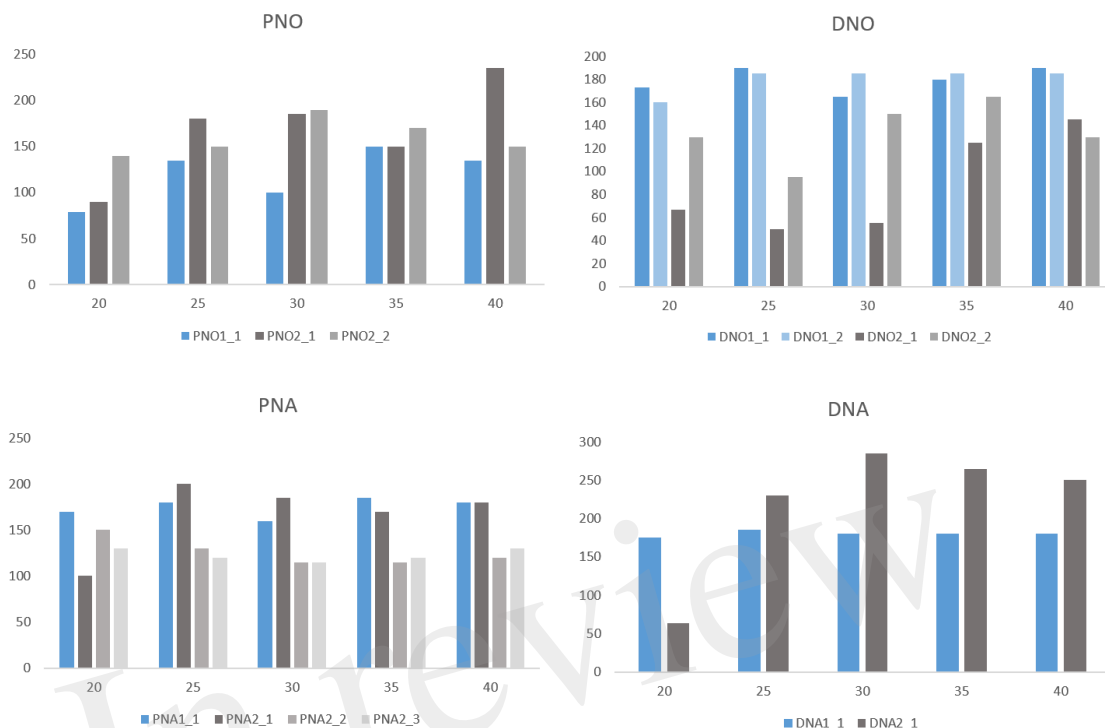


Figure 4. Representation of aggregates' size vs temperature. The results are plotted separately depending on the phosphine and the medium. Blue bars stand for XXX_1 and grey bars for XXX_2 samples.

The infrared spectra of the nanocomposites display the expected bands both from the organometallic complex and from the stabilizing ligands of the NPs. The resulting broadening of the alkynyl moieties are indicative of Au... π interaction between the NPs' surface and the complex.

The UV/vis absorption spectra of the hybrid system present two different signals, as shown in Figure 5 for the hydrophilic nanocomposites with DAPTA and PTA. On the one hand, there is the band around 260 nm attributed to the Au(I) complex. On the other hand, the signal at 500-600 nm is assigned to the characteristic LSPR band of Au NPs. To better study the effect of the quantity of gold NPs added to the formation of hybrid system, absorption spectra of three different systems per organometallic molecule were recorded (DNA_1-3 and PNA_1-3). There is a change in the profile of the more energetically part of the absorption spectra, when the amount of nanoparticles used in the synthesis of the hybrid system increases, there is a loss of the vibrational shape in

the band as an evidence of interaction between NPs and the corresponding organometallic complex.³⁰ Also, there is a slight red shift of 2 nm of the plasmonic band of Au NPs with increasing interactions within the nanocomposite.

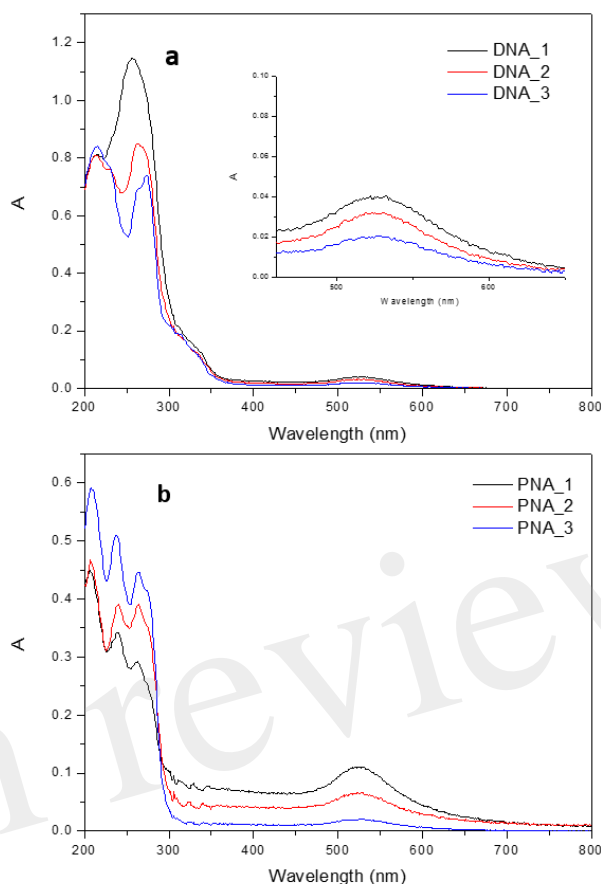


Figure 5. UV-Visible absorption spectra of (a) DNA_1-3 and (b) PNA_1-3 samples.

Biological activity

The biological evaluation was focused on determining the effects against tumour cell growth in HT-29 colon carcinoma and MDA-MB-231 breast cancer (Table 2). Inspection of Table 2 let us retrieve some important conclusions: i) Hybrid organic systems present higher cytotoxicity than the corresponding aqueous systems; ii) hybrid systems present higher cytotoxicity than their respective counterparts, both organometallic complexes and NPs, being a nice cooperative effect than makes promising this encapsulation process regarding the study of their biological activity. However, the toxicity of the drug free organic NPs and the role of CHCl_3 as solvent for redispersion has to be taken into account in future studies.

Table 2. IC₅₀ values of the different hybrid systems and corresponding counterparts.

^a From ref. (Dalton 2014); results of individual experiments are presented

Compound	HT-29 (μM)	MDA-MB-231 (μM)
DNA1	5.85/6.42	> 10
DNA2	> 10/6.40	>10/7.76
PNA1	> 10	> 10
PNA2	> 10	> 10
DNO1	3.69/3.12	2.16/1.29
DNO2	2.99/ 2.53	1.45/> 10
PNO1	1.50/2.13	0.83/1.17
PNO2	3.78/4.53	2.41/1.62
Org NPs	5.30/6.33/4.55	6.21/5.08/6.75
Aqueous NPs	> 10	> 10
AupyPTA ^a	56.09 ± 3.05	52.32 ± 4.76
AypyDAPTA ^a	74.78 ± 9.34	34.07 ± 3.51

Conclusions

The reaction of citrate and oleylamine-capped Au NPs with two different Au(I)-based organometallic complexes gives rise to the successful formation of nanocomposites soluble in either aqueous or organic media, forming large 3D sponge-like aggregates. SEM, SAXS and spectroscopic techniques reveal useful for the verification of interaction between the corresponding building blocks. The lower IC₅₀ values measured for nanocomposites compared to those of individual counterparts evidence their better biological activity against MDA-MB-231 and HT-29 tumor cells, opening a promising area of research in this field.

Authors Contributions

A.F., L.R. and I.O.: Designed the experiments; M.D. and A.P. carried out the synthesis and characterization of hybrid systems. P.L.: carried out biological activity experiments; A. F., L.R. and I.O.: Supervised the project.

All authors wrote and reviewed the manuscript.

Acknowledgements

The authors are grateful to the Ministry of Economy, Industry and Competitiveness of Spain (AEI/FEDER, UE Projects CTQ2016-76120-P and CTQ2015-68370-P). A.F acknowledges financial support from the regional Generalitat de Catalunya authority (2017 SGR 15). A.F is a Serra Húnter fellow. I.O. acknowledges support from DFG (Deutsche Forschungsgemeinschaft). SAXS experiments were performed at the NCD-SWEET beamline of the ALBA Synchrotron Light Facility in collaboration with the ALBA staff.

Supplementary Material

SEM micrographs of nanocomposite samples; DAMMIN low-resolution structures reconstructed from SAXS patterns for nanocomposite samples.

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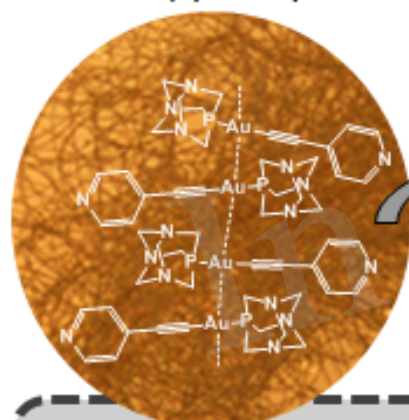
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Figure 1.TIF

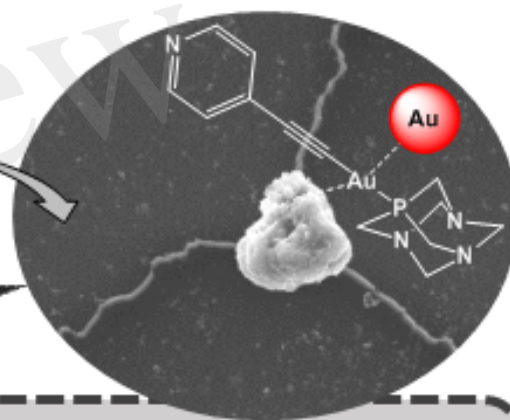
Gold(I) complex



HT-29 colon carcinoma



Hybrid material



MDA-MD-231 breast cancer